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MUTANT CU,ZN SUPEROXIDE DISMUTASE IN MOTOR NEURON DISEASE

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ABSTRACT

Cu,Zn superoxide dismutase (Cu,Zn SOD) is one of several anti-oxidant enzymes which defend the cell against damage by oxygen free radicals. Mutations of the SOD1 gene encoding Cu,Zn SOD are found familial amyotrophic lateral sclerosis, a progressive and fatal paralytic disease which is caused by the death of motor neurons in cortex, brainstem and spinal cord. The disease can be reproduced in transgenic mice by expression of mutant human Cu,Zn SOD. Recent studies both in vitro and in vivo suggest that the effect of mutation is to enhance the generation of oxygen radicals by the mutant enzyme. Thus, mutation converts a protective, anti-oxidant enzyme into a destructive pro-oxidant form which catalyzes free radical damage to which motor neurons are uniquely vulnerable.

INTRODUCTION

It is a great pleasure to acknowledge the contributions of Irwin Fridovich and Joseph McCord to the field of free radical biology at this symposium honoring their receipt of the 1997 Elliott Cresson Medal awarded by the Franklin Institute. The award recognizes their discovery of Cu,Zn superoxide dismutase (Cu,Zn SOD), an enzyme which catalyzes the dismutation of superoxide to hydrogen peroxide. It is one of a series of enzymes which we now recognize protect cells from damage by oxygen free radicals. The formation of such radicals is a "cost" of aerobic metabolism. The two electron reduction of dioxygen by the mitochondrial respiratory chain fuels our energy needs by providing ATP to drive cellular metabolism, but also produces superoxide, a reactive oxygen species with a single unpaired electron. This normally is converted to hydrogen peroxide before it can undergo other free radical reactions, but at some risk. Trace amounts of iron can catalyze the decomposition of hydrogen peroxide to hydroxyl radical, a highly reactive oxygen radical that can damage lipids, proteins or nucleic acids. Cu,Zn SOD in the cytoplasm and Mn SOD in the mitochondrion, provide the first-line cellular defense against oxygen radical-initiated damage.

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DISCUSSION

I want today to talk about the dark side of Cu,Zn SOD. Mutations of the SOD1 gene encoding Cu,Zn SOD are found in patients with a genetic form of a devastating neurological disease (Rosen et al., 1993). The disease is amyotrophic lateral sclerosis. It causes severe paralysis by killing motor neurons in the cortex, brain stem and spinal cord. This leads rapidly to death.

SOD1 Mutations In Familial ALS

The famous French physician, J.M. Charcot, provided the first clinical description of the disease in 1874, while autosomal dominant inheritance of a genetic form of ALS was recognized in the 1950's (e.g., Kurland & Mulder, 1955). The disease is genetically heterogeneous, with perhaps 20% of familial cases associated with mutations of the SOD1 gene (Siddique et al., 1991; Rosen et al., 1993). Currently, more than 50 different point mutations which substitute one amino acid for another have been described in familial ALS. For the most part, these mutations hit the polypeptide backbone of the apoenzyme (Deng et al., 1993). Since the majority of SOD1 mutations in familial ALS are missense mutations, expression of the mutant protein may be required for pathogenesis. This suggests in turn that disease is caused by gain-of-function mutations in SOD1.

Transgenic Models of Familial ALS

The view that the mutant protein may cause disease is reinforced by studies in transgenic mice. Several groups have shown that expression of mutant Cu,Zn SOD at sufficient levels causes motor neuron disease in transgenic mice (Gurney et al., 1994; Ripps et al., 1995; Wong et al., 1995). In all cases, expression of mutant

Cu,Zn SOD causes the death of motor neurons in the spinal cord with consequent paralysis and death (Fig. 1). However, the type of pathology observed in the spinal cord varies with the expression level of mutant Cu,Zn SOD. High levels of expression of either the gly93>ala or gly37>arg mutant forms of Cu,Zn SOD results in primarily vacuolar pathology in ventral horn motor neurons (Dal Canto and Gurney, 1994; Wong et al., 1995). At lower levels of expression, the pathological changes more closely resemble ALS with loss of motor neurons, gliosis, and filamentous pathology including the presence of numerous swollen axons and ubiquitinated Lewy-like bodies in ventral horn neurons (Dal Canto and Gurney, 1995, 1997; Tu et al., 1996).

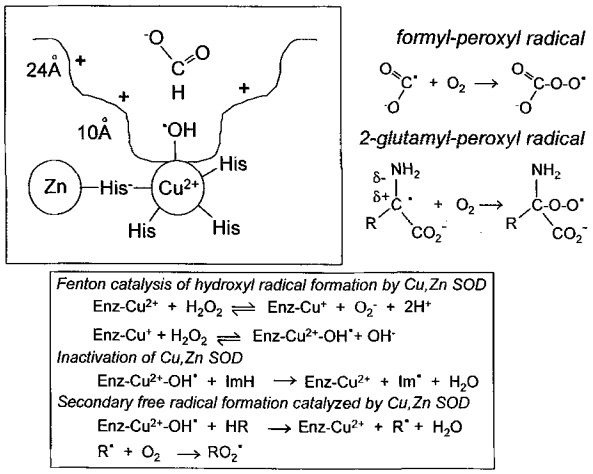

Fig. 1. Formation of secondary oxygen radicals by mutant human Cu,Zn SOD.

Familial ALS Mutations Enhance The Generation Of Oxygen Free Radicals

Clues to the gain of function in mutant Cu,Zn SOD come from an early paper by Ellen Hodgson and Irwin Fridovich (Hodgson & Fridovich, 1975). In that paper they showed that Cu,Zn SOD has minor peroxidase activity. When incubated with hydrogen peroxide, Cu,Zn SOD is slowly and irreversibly inactivated. Several compounds, including xanthine, urate and formate, were shown to protect the enzyme from inactivation by hydrogen peroxide. The data were interpreted to imply that hydrogen peroxide reduced the enzyme bound copper from Cu^{+2} to Cu^{+1}. This allows the Cu^{+1} to react with additional hydrogen peroxide via a Fenton reaction to generate Cu^{+2}-$OH^{\cdot}$. The enzyme subsequently is inactivated by the attack of the bound oxidant on an adjacent histidine (Fig. 1). Compounds which protected the enzyme did so by reacting with the bound oxidant in competition with the adjacent histidine. This experiment suggested that under certain conditions, Cu,Zn SOD could function as a pro-oxidant generator of hydroxyl or other free radicals, rather than solely as an anti-oxidant scavenger of superoxide.

SOD1 mutations found in familial ALS enhance Fenton catalysis by Cu,Zn SOD. Studies by Wiedau-Pazos et al. (1996) and Yim et al. (1996) show that mutant Cu,Zn SOD catalyzes the oxidation of a spin trap by hydrogen peroxide at a higher rate than does the wild-type enzyme. This reaction is dependent on reduction of the active site copper. The deep active site channel in the protein locks away much of the natural chemistry of copper. The active site channel has a shallow surface groove, 24 Å in width, which narrows to 10 Å in the depths of the active site (Fig. 1). The active site channel also contains two lysine and one arginine residues which

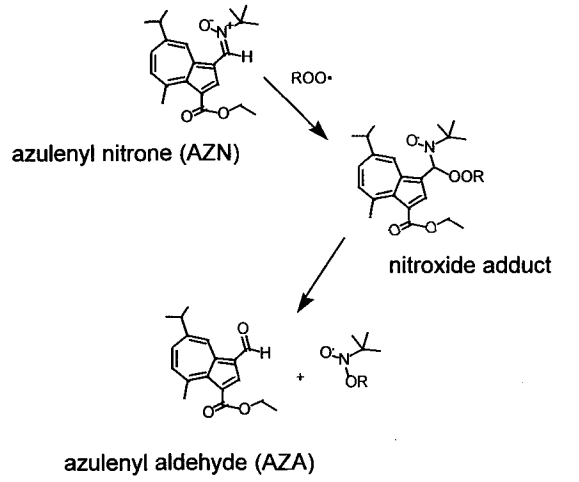

Fig. 2. Reaction of azulenyl nitrone with oxygen radicals.

give it a net positive charge and provide electrostatic attraction for the superoxide substrate (Getzoff et al., 1992). The narrow, positively charged channel normally limits the access of reactants to the active site. Since the SOD1 mutations found in familial ALS hit primarily the polypeptide backbone, they may relax the conformation of the protein and allow the copper catalytic center greater access to solvent. Evidence in support of this hypothesis was provided by Lyons et al. (1996) who show that familial ALS mutations alter the redox behavior of Cu,Zn SOD. This allows reduction of the Cu^{+2} catalytic center to Cu^{+1} at a greater rate than occurs in the wild-type enzyme and provides a mechanism for the enhancement of Fenton catalysis by the mutations found in familial ALS.

Evidence For Enhanced Oxygen Radical Generation Associated With Mutant Cu,Zn SOD Expression In Vivo

Using a novel spin trap, azulenyl nitrone, we explored the tissue content of oxygen free radicals in Cu,Zn SOD transgenic mice (Liu et al., 1997). Trapping of oxygen radicals by azulenyl nitrone (AZN) results as with other nitrones in addition of the radicals to the nitrone double bond to yield nitroxide adducts (Becker, 1996), Fig. 2. These nitroxides, depending upon the type of oxygen radical trapped, can then fragment via a number of pathways to yield the corresponding azulenyl aldehyde

(AZA). The seven-member guiazulenyl ring is a strongly absorbing chromophore which allows the ready detection of AZN and related metabolites by HPLC, while its highly lipophilic character allows it to cross the blood-brain-barrier. This enabled us to sample oxygen radical content in brain and spinal cord of TgN(SOD1-G93A) mice by measuring the AZA/AZN ratio after intravenous injection of the compound.

Reaction of spinal cord extracts from TgN(SOD1-G93A) and TgN(SOD1) mice with AZN in the presence of hydrogen peroxide confirmed the generation of excess oxygen radicals in vitro. Results obtained in vivo were consistent with this result in that an excess of oxygen radical content was seen only in the spinal cord of TgN(SOD1-G93A) mice. TgN(SOD1) mice, which express equivalent levels of wild-type human Cu,Zn SOD, and which do not develop motor neuron disease, did not show an excess in oxygen radical content over control, non-transgenic mice (Liu et al., 1997). Interestingly, salicylate, an in vivo spin trap with selectivity for hydroxyl radical did not detect an increase in oxygen radical content of TgN(SOD1-G93A) mouse spinal cord. This suggests that the increase is likely to be due to the formation of peroxyl radicals. One possibility suggested by this result is that hydroxyl radicals produced by mutant Cu,Zn SOD in vivo may react with membrane lipids to form alkyl or peroxyl radicals too quickly for salicylate to trap them. Alternatively, since small, anionic scavengers protect Cu,Zn SOD from inactivation by reacting with hydroxyl radical within the active site channel (Hodgson & Fridovich, 1975; Yim et al., 1990, 1993), secondary carbon radicals such as formyl or 2-glutamyl radicals could be produced in vivo. Reaction of glutamate with hydroxyl radical within the active site channel could produce a captodative radical ($RC^{\bullet}$) which should react in turn with oxygen to form the corresponding peroxyl radical ($RCOO^{\bullet}$) (Fig. 1). In theory, both lipid peroxyl and 2-glutamyl peroxyl radicals could be trapped by AZN but not by salicylate.

Initiators And Propagators Of Disease In The Tgn(SOD1-G93A) Transgenic Model

Two drug studies in TgN(SOD1-G93A) mice reinforce the view that free radical mechanisms play a role in the transgenic model of motor neuron disease. First, copper chelation therapy with d-penicillamine delays the onset of disease and lengthens survival (Hottinger et al., 1997). Second, dietary supplementation with two antioxidants, vitamin E and selenium, delays disease onset, transiently maintains motor output, but did not extend survival (Gurney et al., 1996). Thus, these observations are consistent with the view that the copper catalytic center of Cu,Zn SOD plays a central role in pathogenesis and that free radical mechanisms initiate disease.

Two other sets of observations also are consistent with the view that free radical mechanisms initiate disease in the transgenic model. First, early pathology in the mouse spinal cord involves most membranous organelles (Dal Canto and Gurney, 1994; Wong et al., 1995; Mourelatos et al., 1996). This is consistent with the initiation of intense peroxidative attack on lipid membranes by an excess of oxygen radical production. Prior to the onset of clinically evident disease, there is swelling of the endoplasmic reticulum, unraveling of mitochondrial structure and fragmentation of the golgi which produce severe vacuolar changes in most ventral horn motor neurons (Chiu et al., 1995; Mourelatos et al., 1996). Second, indices of lipid peroxidation such as formation of malondialdehyde and malondialdhyde protein adducts are increased in TgN(SOD1-G93A) mouse spinal cord (Hall et al., 1997). In addition, protein carbonyl content is increased at end stage disease. Also consistent with the view that free radical mechanisms initiate disease is the minor loss of midbrain dopaminergic neurons in TgN(SOD1-G93A) mice (Kostic et al., 1997).

The Dark Side Of Cu,Zn Superoxide Dismutase

The mutations of the SOD1 gene in familial ALS reveal the dark side of Cu,Zn SOD. A normally minor peroxidase activity which generates hydroxyl radical via the Fenton reaction is enhanced by mutation to such an extent that it now causes the death of selectively vulnerable neurons. We are in the early stages of unraveling pathogenic mechanisms in the disease, although that effort will proceed much more quickly due to the availability of a transgenic model in which to test hypotheses. However, mysteries abound. We only incompletely understand the link between free radical damage and propagation of glutamate excitotoxicty. The selective vulnerability of motor neurons also remains a mystery. Hopefully, more complete understanding of pathogenic mechanisms will translate into the discovery of effective therapeutics for this devastating neurodegenerative disease.

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